



## **Application of the Ceditest FMDV type O and FMDV-NS enzyme-linked immunosorbent assays for detection of antibodies against Foot-and-mouth disease virus in selected livestock and wildlife species in Uganda**

**Ayebazibwe, Chrisostom; Mwiine, Frank Norbert; Balinda, Sheila Nina; Tjørnehøj, Kirsten; Alexandersen, Søren**

*Published in:*  
Journal of Veterinary Diagnostic Investigation

*Link to article, DOI:*  
[10.1177/1040638711435807](https://doi.org/10.1177/1040638711435807)

*Publication date:*  
2012

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
Ayebazibwe, C., Mwiine, F. N., Balinda, S. N., Tjørnehøj, K., & Alexandersen, S. (2012). Application of the Ceditest FMDV type O and FMDV-NS enzyme-linked immunosorbent assays for detection of antibodies against Foot-and-mouth disease virus in selected livestock and wildlife species in Uganda. *Journal of Veterinary Diagnostic Investigation*, 24(2), 270-276. <https://doi.org/10.1177/1040638711435807>

---

### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

# Journal of Veterinary Diagnostic Investigation

<http://vdi.sagepub.com/>

---

## Application of the Ceditest® FMDV type O and FMDV-NS enzyme-linked immunosorbent assays for detection of antibodies against *Foot-and-mouth disease virus* in selected livestock and wildlife species in Uganda

Chrisostom Ayebazibwe, Frank Norbert Mwiine, Sheila Nina Balinda, Kirsten Tjørnehøj and Søren Alexandersen

*J VET Diagn Invest* 2012 24: 270

DOI: 10.1177/1040638711435807

The online version of this article can be found at:

<http://vdi.sagepub.com/content/24/2/270>

---

Published by:



<http://www.sagepublications.com>

On behalf of:



Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc.

Additional services and information for *Journal of Veterinary Diagnostic Investigation* can be found at:

**Email Alerts:** <http://vdi.sagepub.com/cgi/alerts>

**Subscriptions:** <http://vdi.sagepub.com/subscriptions>

**Reprints:** <http://www.sagepub.com/journalsReprints.nav>

**Permissions:** <http://www.sagepub.com/journalsPermissions.nav>

>> [Version of Record](#) - Feb 29, 2012

[What is This?](#)

# Application of the Ceditest® FMDV type O and FMDV-NS enzyme-linked immunosorbent assays for detection of antibodies against *Foot-and-mouth disease virus* in selected livestock and wildlife species in Uganda

Chrisostom Ayebazibwe, Frank Norbert Mwiine, Sheila Nina Balinda, Kirsten Tjørnehøj,<sup>1</sup> Søren Alexandersen

**Abstract.** Diagnosis and control of *Foot-and-mouth disease virus* (FMDV) requires rapid and sensitive diagnostic tests. Two antibody enzyme-linked immunosorbent assay (ELISA) kits, Ceditest® FMDV-NS for the detection of antibodies against the nonstructural proteins of all FMDV serotypes and Ceditest® FMDV type O for the detection of antibodies against serotype O, were evaluated under African endemic conditions where the presence of multiple serotypes and the use of nonpurified vaccines complicate serological diagnosis. Serum samples from 218 African buffalo, 758 cattle, 304 goats, and 88 sheep were tested using both kits, and selected samples were tested not only in serotype-specific ELISAs for antibodies against primarily FMDV serotype O, but also against other serotypes. The FMDV-NS assay detected far more positive samples (93%) than the FMDV type O assay (30%) in buffalo ( $P < 0.05$ ), with predominant antibodies against the South African Territories (SAT) serotypes, while the seroprevalence was generally comparable in cattle with antibodies against serotype O elicited by infection and/or vaccination. However, some districts had higher seroprevalence using the FMDV type O assay indicating vaccination without infection, while 1 cattle herd with antibodies against the SAT serotypes had far more positive samples (85%) using the FMDV-NS versus the FMDV type O (10%), consistent with the latter test's lower sensitivity for antibodies against SAT serotypes. Based on the current investigation, the FMDV type O ELISA may be limited by the presence of SAT serotypes. The FMDV NS assay worked well as a screening test for antibodies against all FMDV serotypes present in Uganda; however, as long as nonpurified vaccines are applied in the region, this test cannot be used to differentiate between vaccinated and infected animals.

**Key words:** Ceditest® FMDV-NS; Ceditest® FMDV type O; Foot-and-mouth disease; Uganda.

## Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals including domestic ruminants, pigs, and more than 70 wild animal species.<sup>1,21</sup> *Foot-and-mouth disease virus* (FMDV; order *Picornavirales*, family *Picornaviridae*, genus *Aphthovirus*) contains 7 serotypes, namely O, A, C, Asia1, Southern African Territories (SAT)1, SAT2, and SAT3, all with a number of subtypes. Infection with 1 serotype does not prevent infection with other serotypes.<sup>8</sup> Different serotypes have distinct geographical distributions and epidemiology; however, they are clinically indistinguishable.<sup>22</sup>

The epidemiology of FMD in East Africa is complex with 5 (O, A, SAT1, SAT2, and SAT3) out of the 7 serotypes being continuously present.<sup>22</sup> Serotype C was last diagnosed in

Kenya in 2004<sup>18</sup> while serotype Asia1 has never been diagnosed on the African continent.<sup>22</sup>

Previously, serological diagnosis of FMDV implied running up to 7 different tests for antibodies against each of the relevant serotypes; however, more broadly directed antibody tests detecting antibodies against all 7 serotypes of FMDV

---

From Ministry of Agriculture, Animal Industry and Fisheries, Entebbe, Uganda (Ayebazibwe, Mwiine), Makerere University Institute of Environment and Natural Resources, Kampala, Uganda (Balinda), and National Veterinary Institute, Technical University of Denmark, Lindholm, Denmark (Tjørnehøj, Alexandersen).

<sup>1</sup>Corresponding Author: Kirsten Tjørnehøj, National Veterinary Institute, Technical University of Denmark, Lindholm, DK 4771 Kalvehave, Denmark. kitj@vet.dtu.dk

have recently been offered by a number of commercial companies, including an enzyme-linked immunosorbent assay (ELISA) kit for detection of antibodies against all 7 serotypes of FMDV marketed by Prionics AG, Switzerland: the Ceditest® FMDV-NS<sup>a</sup> (hereafter, NSP test). The same company has marketed a screening test for detection of antibodies against serotype O, the Ceditest® FMDV type O ELISA kit<sup>b</sup> (hereafter, SP-O test), which has such broad specificities for antibodies against the Eurasian serotypes of FMDV (O, A, C, and Asia1)<sup>9</sup> that it has been speculated that this test could work as a pan-FMDV antibody test.

The NSP test is a blocking ELISA that detects antibodies against the nonstructural 3ABC protein of FMDV in infected animals, independent of the serotype that induced the antibodies.<sup>19</sup> Like other commercially available test kits for antibodies against the nonstructural proteins (NSPs) of FMDV, the NSP assay has important applications in differentiating infected from vaccinated animals.<sup>19</sup> Conversely, the SP-O assay is based on the structural proteins (SPs) of FMDV serotype O and can be used in cattle, sheep, pigs, and goats.<sup>9</sup> These 2 test kits have been successfully used for investigating FMD outbreaks and for control programs.<sup>9,16,17</sup>

The NSP ELISAs are widely applied in the diagnosis of FMD and have been evaluated in Europe<sup>5</sup> and to a lesser extent in Africa.<sup>6,7</sup> In a direct comparison of 6 NSP ELISAs, the NSP test had comparative sensitivities of 98.9% and 96.6% in field sera from Israel and Zimbabwe, respectively, while diagnostic specificity was 97.2% in nonvaccinated animals and 99.5% in experimental animals that had received 1 vaccination.<sup>5</sup> In Africa, using Hui–Walter latent class modeling and comparing with a combination of neutralization test results for all 3 SAT serotypes of FMDV, it has been demonstrated that the NSP test is a useful tool for screening buffalo infected with multiple SAT serotypes of FMDV with estimated sensitivity of 87.7% and specificity of 87.3%.<sup>6</sup> Using the same modeling methodology but without comparing to neutralization test results, estimated sensitivity and specificity of 96.6% and 90.9%, respectively, have been found in Zebu cattle kept under extensive rangeland conditions in Cameroon.<sup>7</sup> Although only limited studies of performance of these tests have been carried out among sheep and goats, lower sensitivity has been reported particularly in sheep.<sup>13</sup>

The SP-O test has mainly been used and evaluated in Europe and has been found to identify antibodies against serotypes O, A, C, and Asia1 in cattle, with higher sensitivity for antibodies against serotype O.<sup>9</sup> The aim of the present study was to evaluate the performance of the SP-O and NSP tests in Ugandan cattle, sheep, goats, and African buffalo (*Syncerus caffer*). In addition, the SP-O test was compared to a solid phase blocking ELISA (SPBE) for detection of antibodies against FMDV serotype O.<sup>4</sup>

## Materials and methods

Serum samples were collected from Ugandan African buffalo, cattle, goats, and sheep. Two hundred eighteen

samples from African buffalo were collected from Lake Mburo, Kidepo Valley, Murchison Falls, and Queen Elizabeth National Parks as described elsewhere.<sup>2,3</sup> In addition, 449 cattle sera were collected in July 2006 during an extensive postoutbreak (PO) sampling in a number of districts in the western and southwestern regions of Uganda and 309 sera were collected during an attempted random sampling (RS) within Kasese and Bushenyi Districts in 2007.<sup>14,15</sup> Three hundred four goat sera and 88 sheep sera were collected from the same farms or villages during these sampling trips.<sup>4</sup>

Testing for antibodies against FMDV was done using the NSP<sup>a</sup> and SP-O<sup>b</sup> tests according to the manufacturer's instructions. Optical density values (OD) were determined using a dual wavelength ELISA reader<sup>c</sup> at 620 nm and 450 nm. Results were expressed as OD% (ODP: OD<sub>sample</sub> in percent of OD<sub>negative controls</sub>),<sup>4</sup> and cutoffs were set at 50%.

Selected samples, mostly positive for antibodies against NSPs, were screened at dilution 1:5 using an in-house SPBE for antibodies against FMDV serotype O (SPBE-O) with a cutoff of 50% as previously described.<sup>4</sup> The selected samples included 174 buffalo sera, 126 cattle sera from the 2006 PO sampling, and 81 cattle sera from the 2007 RS sampling. The selected cattle sera constituted 28–80% of sera from each of the 18 herds with a history of recent clinical signs of FMD in 2006 and 17–100% of sera from 16 randomly sampled farms without clinical signs of FMD in 2007. For small ruminants, all samples positive in the NSP test and/or the SP-O test from both 2006 and 2007 were further tested using the SPBE-O. In addition, 20 sera from 1 herd (Ks12)<sup>15</sup> were examined in SPBEs for antibodies against all 7 serotypes of FMDV as previously described.<sup>4</sup> Differences between groups were tested for statistical significance using chi-square, and differences between seroprevalence obtained with the NSP and SP-O tests were analyzed using McNemar test in a spreadsheet program.<sup>d</sup>

## Results

Antibodies against NSPs, and SPs of FMDV serotype O, were detected in African buffalo, cattle, goat, and sheep samples (Table 1). Seroprevalence of antibodies against NSP was much higher in African buffalo (93%) compared to cattle (48%), sheep (28%), and goats (9%), while cattle had the highest number of positives in the SP-O test (52%) compared to buffalo (30%) sheep (24%), and goats (7%).

In buffalo, for both tests, there was no significant difference in the seroprevalence of FMDV antibodies between national parks ( $P = 0.758$  and  $P = 0.413$  for the NSP and SP-O tests, respectively). Thus, the data from all buffalo samples were pooled, and statistical analysis of these results confirmed that the NSP test detected significantly more seropositive buffalo than the SP-O test ( $P < 0.005$ ) in all the 4 investigated national parks.

The results were less clear in cattle because the NSP test detected significantly more positive samples than did the SP-O test in samples from the 2007 RS, while it detected significantly fewer samples than did the SP-O test in the 2006

**Table 1.** Comparative evaluation of Ceditest® FMDV-NS (NSP) and Ceditest® FMDV type O (SP-O) enzyme-linked immunosorbent assay for the detection of antibodies against nonstructural and structural proteins of *Foot-and-mouth disease virus* in livestock and wildlife species in various districts and national parks in Uganda.

Samples/Origin	Total	NSP+	SP-O+	NSP+/SP-O+	NSP+/SP-O–	NSP–/SP-O+	NSP–/SP-O–
Buffalo							
2001–2008							
MFNP	53	51 (96)	19 (36)	18	33	1	1
LMNP	18	17 (94)	6 (33)	6	11	0	1
KVNP	39	36 (92)	14 (36)	13	23	1	2
QENP	108	99 (92)	27 (25)	27	72	0	9
Total	218	203 (93)	66 (30)	64	139	2	13
Cattle							
PO, 2006							
Bushenyi	59	28 (48)	30 (51)	28	0	2	29
Isingiro	11	8 (73)	11 (91)	7	1	3	0
Kasese	203	142 (70)	142 (70)	122	21	20	40
Mbarara	51	36 (71)	48 (94)	35	1	13	2
Mpigi	23	18 (78)	21 (91)	17	1	4	1
Kiruhura	87	7 (8)	50 (58)	6	1	44	36
Kampala	15	1 (7)	4 (27)	0	1	4	10
Subtotal	449	240 (53)	306 (68)	215	26	90	118
RS, 2007							
Kasese	193	118 (61)	83 (43)	83	35	0	75
Bushenyi	116	4 (4)	5 (4)	0	5	4	107
Subtotal	309	122 (39)	88 (29)	83	40	5	181
Total	758	362 (48)	394 (52)	298	66	95	299
Goats							
PO, 2006							
Kasese	9	3 (33)	6 (67)	3	0	3	3
Bushenyi	15	0 (0)	0 (0)	0	0	0	15
Mbarara	4	4 (100)	4 (100)	4	0	0	0
Isingiro	5	0 (0)	0 (0)	0	0	0	5
Subtotal	33	7 (21)	10 (30)	7	0	3	23
RS, 2007							
Kasese	143	20 (14)	9 (6)	4	16	2	121
Bushenyi	128	0 (0)	1 (1)	0	1	3	124
Subtotal	271	20 (7)	10 (4)	4	17	5	245
Total	304	27 (9)	20 (7)	11	17	8	268
Sheep							
PO, 2006							
Kasese	13	13 (100)	13 (100)	13	0	0	0
Subtotal	13	13 (100)	13 (100)	13	0	0	0
RS, 2007							
Kasese	57	12 (21)	8 (14)	6	6	2	43
Bushenyi	18	0 (0)	0 (0)	0	0	0	18
Subtotal	75	12 (16)	8 (11)	6	6	2	61
Total	88	25 (28)	21 (24)	19	6	2	61

\*+ = positive; – = negative; PO = postoutbreak sampling; RS = random sampling; MFNP: Murchison Falls National Park; LMNP: Lake Mburo National Park; KVNP: Kidepo Valley National Park; QENP: Queen Elizabeth National Park. Numbers in parentheses are percentages.

PO sampling ( $P < 0.001$  for both comparisons). The difference in 2007 was caused by more positive samples in the NSP test than in the SP-O test in the Kasese District ( $P < 0.001$ ), while in 2006, it was caused by more positive samples in the SP-O test than in the NSP test in Mbarara ( $P =$

0.013) and Kiruhura ( $P < 0.001$ ) districts. Moreover, the 2 tests had different outcomes for approximately 20% of the samples from Kasese District in 2006, but this difference was not statistically significant since the samples that were only positive in one of the tests were distributed equally

**Table 2.** Comparative screening results for *Foot-and-mouth disease virus* using the Ceditest® FMDV type O (SP-O) enzyme-linked immunosorbent assay (ELISA) and the solid phase blocking ELISA (SPBE-O) in buffalo, cattle, and small ruminants in Uganda.

Species	Total	NSP positive	Results of the SP-O/SPBE-O ELISAs			
			+/+	+/-	-/+	-/-
Buffalo	174	169	47	6	79	42
Postoutbreak sampling, 2006						
Cattle	126	120	106	2	0	18
Goats	10	7	7	3	0*	0*
Sheep	13	13	13	0	0*	0*
Random sampling, 2007						
Cattle	81	76	50	1	16	14
Goats	26	21	5	4	4	13
Sheep	14	12	7	1	2	4

\*NSP = Ceditest® FMDV-NS (Cedi Diagnostics B.V., Leystad, The Netherlands [currently produced and marketed as Priocheck® FMDV-NS by Prionics AG, Schlieren-Zurich, Switzerland]. + = positive; - = negative. The samples investigated by SPBE-O were all SP-O positive.

between the 2 tests (and thus not significantly different,  $P = 0.859$ ). The remaining district samples (Bushenyi, Isingiro, Mpigi, and Kampala in 2006, and Bushenyi in 2007) had comparable results with only a few samples differing between the 2 tests.

With regard to sera from goats and sheep, the differences between the results obtained with the 2 tests were either inconclusive or insignificant apart from those of goats in 2007 where the NSP test found more positive animals than did the SP-O test ( $P = 0.0105$ ).

When comparing the results obtained in dilution 1:5 in the SPBE-O test to results in the SP-O test, there was generally good agreement between the results obtained with the 2 tests in cattle, sheep, and goats in the 2006 PO sampling, while the results were more scattered in the 2007 RS and in buffalo, where the SPBE-O screening ELISA identified more positive cattle and buffalo samples than did the SP-O test (Table 2).

In the 2006 PO sampling, on-farm seroprevalence in the NSP and SP-O tests were generally comparable<sup>14</sup>; however, 1 out of 22 cattle herds (Ks12) with a history of recent clinical signs of FMD had much higher prevalence of antibodies against FMDV in the NSP test than in the SP-O test.<sup>14</sup> Analysis of serotype-specificity of FMDV antibodies of 20 heads of cattle from this herd using SPBEs for all 7 serotypes revealed that these cattle exclusively had antibodies against the SAT serotypes (Table 3). Most of these 20 samples had low antibody titers against serotypes SAT2 and SAT3; however, 1 animal had a titer of 80 for antibodies against SAT2, 3 animals had titers of 80–160 against SAT3, and 1 animal had a titer of 640 against SAT1. This herd was regularly grazed in Queen Elizabeth National Park.

## Discussion

Interpretation of the higher seroprevalence of antibodies against NSPs than against SPs of FMDV serotype O in the

investigated buffalo sera should take into consideration that when titrated, these sera had higher seroprevalences of antibodies against the FMDV SAT serotypes than against serotype O.<sup>2,3</sup> Thus, these data suggest that the SP-O test may be much less sensitive for antibodies against the SAT serotypes of FMDV circulating in Uganda than the NSP test. It may be speculated that the low sensitivity of the SP-O test in buffalo could be species-related; however, this test is a blocking ELISA, which is generally not species-specific. Moreover, the similar finding in a cattle herd (Ks12) of much higher seroprevalence in the NSP test and in the SPBEs for antibodies against the SAT serotypes than in both the SP-O and SPBE-O tests supports that the low sensitivity in buffalo was not species-related.

In cattle, the high seroprevalence in both tests in most districts in 2006 were found during a major outbreak of FMDV serotype O in the region in 2006, which was preceded and followed by vaccination campaigns with nonpurified trivalent vaccines containing FMDV serotypes O, SAT1, and SAT2. Moreover, the majority of the sampled herds exhibited clinical evidence of exposure to FMDV and/or reported previous vaccination, and a serotype O virus was isolated from 2 probang samples collected from this outbreak.<sup>15</sup> In agreement with this, all herds with clinical evidence of FMDV infection, except the 1 Kasese herd described in Table 3 (Ks12), had high seroprevalence in the SP-O test and had high titers of antibodies against serotype O in the SPBE-O ELISA.<sup>15</sup> The higher seroprevalence registered with the SP-O test in Mbarara, Kiruhura, and Kampala districts is consistent with vaccinated animals that have not recently been infected.

The Kasese herd (Ks12; Table 3) with much higher seroprevalence in the NSP test (85%) than in the SP-O test (10%) had serological evidence of exposure to SAT serotypes, while all tested samples were negative in the SPBE-O ELISA. Thus, this result supports the preceding conclusion



**Table 3.** Serological analysis for *Foot-and-mouth disease virus* (FMDV) of a cattle herd (Ks12) in the Kasese District of Uganda with high seroprevalence in the Ceditest® FMDV-NS enzyme-linked immunosorbent assay (ELISA) and low seroprevalence in the Ceditest® FMDV type O ELISA.\*

Sample ID	Optical density percentage in screening for antibodies against:		SPBE antibody titer for FMDV serotype			
	FMDV-NS	FMDV type O	O	SAT1	SAT2	SAT3
53	<b>30</b>	69	ND	–	<b>40</b>	20
55	<b>6</b>	2	–	ND	ND	ND
56	<b>35</b>	83	–	–	<b>20</b>	10
57	<b>29</b>	88	–	<b>10</b>	10	ND
58	<b>7</b>	83	–	–	<b>40</b>	10
62	<b>7</b>	96	–	ND	ND	ND
63	<b>43</b>	56	–	–	10	<b>40</b>
64	<b>41</b>	90	–	–	20	<b>20</b>
67	<b>20</b>	107	–	–	20	–
69	<b>27</b>	104	–	<b>640</b>	20	10
70	<b>11</b>	71	–	INC	40	10
71	<b>35</b>	99	–	ND	ND	ND
72	52	97	–	<b>20</b>	10	<b>20</b>
73	<b>27</b>	99	–	–	ND	ND
74	<b>47</b>	83	–	ND	ND	ND
75	96	23	–	–	10	<b>80</b>
76	51	107	–	–	<b>20</b>	ND
78	<b>18</b>	94	–	–	<b>20</b>	INC
79	<b>40</b>	71	–	INC	80	<b>160</b>
82	<b>5</b>	82	–	–	40	<b>160</b>
Total	17/20 (85)	2/20 (10)	0/19 (0)	3/16 (19)	15/15 (100)	11/13 (85)

\*SPBE = solid phase blocking enzyme-linked immunosorbent assay; SAT = South African Territories. Positive samples (optical density percentage < 50%) are in bold type. The highest titer in each sample is in bold italic type. – = negative when screened in a dilution of 1:10; ND = not done due to depletion of serum; INC = inconclusive, which means positive when screened in a dilution of 1:10, but negative in a dilution of 1:10 (just above the cut-off), when titrated from 1:10 to 1:1280. Total indicates number of positive/number of tested, with percentages in parentheses.

from the buffalo data that the SP-O test is a less suitable tool for screening antibodies against FMDV than the NSP test when antibodies against the Ugandan SAT serotypes of FMDV are prevailing.

The 2007 RS differed from the 2006 PO sampling in that the 2 districts under study had very different levels of seroprevalence in both the NSP and SP-O tests. Moreover, Bushenyi District had very low seroprevalence and had, except for a small, quickly contained outbreak in 2006 (sampled in the 2006 PO sampling), been free from FMD for a prolonged time,<sup>14</sup> while Kasese District had high seroprevalence reflecting a situation with frequent reports of FMD outbreaks and vaccinations.

Thirty-five of the Kasese 2007 samples (18%) were positive only in the NSP test (Table 1), and half of these samples came from a herd with higher titers of antibodies against the SAT serotypes than against serotype O (K1),<sup>14</sup> confirming the results from Ks12 sampled in 2006, while the remaining 18 samples were dispersed among 10 of the 16 sampled herds.<sup>14</sup>

The NSP test, like other commercially available test kits for antibodies against the NSPs of FMDV, have important applications in differentiating infected from vaccinated animals,<sup>19</sup> but this usage may be complicated by nonpurified FMDV vaccines inducing antibodies against the NSPs.<sup>10,14,20</sup> Such vaccines including FMDV serotypes O, SAT1, and SAT2 were applied in the investigated regions in 2005, 2006, and 2007, and it is thus not possible to definitively conclude if the antibodies detected in the NSP test were elicited by vaccination and/or infection.

The differences between the performances of the NSP test and the SP-O test in small ruminants followed the results in cattle, but were not statistically significant due to low numbers of positives. These variations were most likely results of the presence of antibodies against FMDV serotype O after the outbreak of FMDV serotype O during 2006.<sup>4</sup> The much higher seroprevalence in sheep and goats in the 2006 PO sampling than in the 2007 RS, combined with the higher seroprevalence in cattle in 2007, may indicate faster waning of antibodies in these species than in cattle as previously

reported,<sup>11,12</sup> but could also be a consequence of the common practice of not vaccinating small ruminants.

The results of the SPBE-O test matched those of the SP-O test in cattle sampled in 2006 (PO sampling), while the first test was more sensitive in cattle sampled in 2007 RS and in buffalo that tested positive for antibodies against SAT serotypes. In small ruminants, these 2 tests were also more in agreement in the 2006 PO sampling than in the 2007 RS. The results from buffalo clearly demonstrate that the SPBE-O when used in dilution of 1:5 is less specific with regard to identifying serotype than the SP-O test. The data, taken together with reported predominance of antibodies against SAT serotypes,<sup>2,3</sup> indicate that the SP-O test may not be very efficient for identification of antibodies against the SAT serotypes of FMDV, and thus cannot be recommended as a pan-FMDV antibody test.

Like other commercially available test kits for antibodies against the NSPs of FMDV, the NSP test has important applications in differentiating infected from vaccinated animals,<sup>21</sup> but this usage may be complicated by detection of antibodies against FMDV NSPs elicited by nonpurified FMDV vaccines,<sup>10,21</sup> which are applied in some sub-Saharan African countries including Uganda. This was also the case in the samplings in the current study, where nonpurified trivalent vaccines had been used in the area in vaccination campaigns in cattle in 2005, 2006, and 2007. Thus, in the presented analysis of cattle samples from 2006 and 2007, it is impossible to conclude whether the identified antibodies were induced by vaccination and/or infection. However, the NSP test can still be applied for mass screening to identify animals with antibodies against FMDV whether these are vaccine-derived or elicited by infection.<sup>20</sup>

In conclusion, though the SP-O test is of low specificity toward antibodies against the Eurasian FMDV serotypes<sup>9</sup> and it has been speculated that this test could be used as a pan-FMDV antibody test, this usage cannot be recommended in populations where the SAT serotypes of FMDV are prevalent, as exemplified by the SAT-infected buffalo and the cattle herd exposed to 1 or more of the SAT serotypes, only. The SP-O test can be used in combination with the NSP test when investigating FMD outbreaks caused by serotype O; however, it will not be possible to use the NSP test to differentiate between vaccinated and infected animals as long as nonpurified vaccines are applied in the region.

### Acknowledgements

Chrisostom Ayebazibwe, Frank Norbert Mwiine, and Sheila Nina Balinda equally contributed to this research. The authors thank the Ministry of Agriculture Animal Industry and Fisheries, Uganda, for allowing us to undertake this study.

### Sources and manufacturers

a. Ceditest® FMDV-NS, Cedi Diagnostics B.V., Leystad, The Netherlands (currently produced and marketed as Priocheck® FMDV-NS by Prionics AG, Schlieren-Zurich, Switzerland).

- b. Ceditest® FMDV-type O, Cedi Diagnostics B.V., Leystad, The Netherlands (currently produced and marketed as Priocheck® FMDV-type O by Prionics AG, Schlieren-Zurich, Switzerland).
- c. Thermo Labsystems Multiskan Ascent® Photometric plate reader, Thermo Fischer, Slangstrup, Denmark.
- d. Microsoft® Office Excel® 2007, Microsoft Corp., Redmond, WA.

### Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was funded by the Danish International Development Agency under the Livestock Wildlife Diseases in East Africa Project (grant no. P104.Dan.8.1.316).

### References

1. Alexandersen S, Mowat N: 2005, Foot-and-mouth disease: host range and pathogenesis. *Curr Top Microbiol Immunol* 288:9–42.
2. Ayebazibwe C, Mwiine FN, Balinda SN, et al.: 2010, Antibodies against foot-and-mouth disease (FMD) virus in African buffaloes (*Syncerus caffer*) in selected National Parks in Uganda (2001–2003). *Transbound Emerg Dis* 57:286–292.
3. Ayebazibwe C, Mwiine FN, Tjørnehøj K, et al.: 2010, The role of African buffaloes (*Syncerus caffer*) in the maintenance of foot-and-mouth disease in Uganda. *BMC Vet Res* 6:54.
4. Balinda SN, Tjørnehøj K, Muwanika VB, et al.: 2009, Prevalence estimates of antibodies against foot-and-mouth disease virus in small ruminants in Uganda. *Transbound Emerg Dis* 56:362–371.
5. Brocchi E, Bergmann IE, Dekker A, et al.: 2006, Comparative evaluation of six ELISAs for detection of antibodies to the non-structural proteins of foot-and-mouth disease virus. *Vaccine* 24:6966–6979.
6. Bronsvoort BMC, Parida S, Handel I, et al.: 2008, Serological survey for foot-and-mouth disease in wildlife in eastern Africa and estimation of test parameters of a nonstructural protein enzyme-linked immunosorbent assay for buffalo. *Clin Vaccine Immunol* 15:1003–1011.
7. Bronsvoort BMC, Toft N, Bergmann IE, et al.: 2006, Evaluation of three 3ABC ELISAs for foot-and-mouth disease non-structural antibodies using latent class analysis. *BMC Vet Res* 2:30.
8. Cartwright B, Chapman WG, Sharpe RT: 1982, Stimulation by heterotypic antigens of foot-and-mouth disease virus in vaccinated cattle. *Res Vet Sci* 32:338–342.
9. Chénard GK, Miedema K, Moonen P, et al.: 2003, A solid-phase blocking ELISA for detection of type O foot-and-mouth disease virus antibodies suitable for mass serology. *J Virol Methods* 107:89–98.



10. Clavijo A, Wright P, Kitching P: 2004, Developments in diagnostic techniques for differentiating infection from vaccination in foot-and-mouth disease. *Vet J* 167:9–22.
11. Cunliffe HR: 1964, Observations on the duration of immunity in cattle after experimental infection with foot-and-mouth disease. *Cornell Vet* 54:501–510.
12. Dellers RW, Hyde JL: 1964, Response of sheep to experimental infection with foot-and-mouth disease virus. *Am J Vet Res* 25:469–473.
13. Kitching RP: 2002, Identification of foot-and-mouth disease virus carrier and subclinically infected animals and differentiation from vaccinated animals. *Rev Sci Tech* 21:531–538.
14. Mwiine FN, Ayebazibwe C, Olaho-Mukani W, et al.: 2010, Prevalence of antibodies against foot-and-mouth disease virus in cattle in Kasese and Bushenyi districts in Uganda. *Int J Anim Vet Adv* 2:89–96.
15. Mwiine FN, Ayebazibwe C, Olaho-Mukani W, et al.: 2010, Serotype-specificity of antibodies against foot-and-mouth disease virus in cattle in selected districts in Uganda. *Transbound Emerg Dis* 57:365–374.
16. Paton DJ, de Clercq K, Greiner M, et al.: 2006, Application of non-structural protein antibody tests in substantiating freedom from foot-and-mouth disease virus infection after emergency vaccination of cattle. *Vaccine* 24:6503–6512.
17. Paton DJ, Ferris NP, Hutchings GH, et al.: 2009, Investigations into the cause of foot-and-mouth disease virus seropositive small ruminants in Cyprus during 2007. *Transbound Emerg Dis* 56:321–328.
18. Sangula AK, Siegismund HR, Belsham GJ, et al.: 2011, Low diversity of foot-and-mouth disease serotype C virus in Kenya: evidence for probable vaccine strain re-introductions in the field. *Epidemiol Infect* 139:189–196.
19. Sørensen KJ, Madsen KG, Madsen ES, et al.: 1998, Differentiation of infection from vaccination in foot-and-mouth disease by detection of antibodies to the non-structural proteins 3D, 3AB and 3ABC in ELISA using antigens expressed in baculovirus. *Arch Virol* 143:1461–1476.
20. Suttmoller P, Barteling SS, Olascoaga RC, Sumption KJ: 2003, Control and eradication of foot-and-mouth disease. *Virus Res* 91:101–144.
21. Thomson GR: 1994, Foot-and-mouth disease. *In: Infectious disease of livestock with special reference to southern Africa*, ed. Coetzer JAW, Thomson GR, Tustin RC, Kriek NPJ, 1st ed., pp. 825–852. Oxford University Press, Cape Town, South Africa.
22. Vosloo W, Bastos AD, Sangare O, et al.: 2002, Review of the status and control of foot and mouth disease in sub-Saharan Africa. *Rev Sci Tech* 21:437–449.